L Number	Hits	Search Text	DB	Time stamp
1	50	(thr-x-tyr) or (thr-pro-tyr)	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:18
3	0	((thr-x-tyr) or (thr-pro-tyr)) same immunoassay	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:18
2	20	((thr-x-tyr) or (thr-pro-tyr)) and immunoassay	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:18

L Number	Hits	Search Text	DB	Time stamp
1	199	dual adj phosphorylation	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:36
2	66	(dual adj phosphorylation) same substrate	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:36
3	21	((dual adj phosphorylation) same substrate) same antibod$	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:36
4	195082	(test adj compound) or modulator or (drug adj candidate)	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:38
5	21	(((dual adj phosphorylation) same substrate) same antibod$) and ((test adj compound) or modulator or (drug adj candidate))	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:38

L Number	Hits	Search Text	DB	Time stamp
1	199	dual adj phosphorylation	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:36
2	66	(dual adj phosphorylation) same substrate	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:36
3	21	((dual adj phosphorylation) same substrate) same antibod$	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:36
4	195082	(test adj compound) or modulator or (drug adj candidate)	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:38
5	21	(((dual adj phosphorylation) same substrate) same antibod$) and ((test adj compound) or modulator or (drug adj candidate))	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:45
6	2600	synthetic adj substrate	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:45
7	2	(dual adj phosphorylation) and (synthetic adj substrate)	USPAT; US-PGPUB; EPO; DERWENT	2003/11/24 13:45
8	0	(synthetic adj substrate) same mkk	USPAT; US-PGPUB	2003/11/24 13:47
9	1	(synthetic adj substrate) and sapk	USPAT; US-PGPUB	2003/11/24 13:47
10	14	(synthetic adj substrate) and jnk	USPAT; US-PGPUB	2003/11/24 13:50
11	0	((dual adj phosphorylation) same substrate) same immunoassay	USPAT; US-PGPUB	2003/11/24 13:50
12	13	((dual adj phosphorylation) same substrate) and immunoassay	USPAT; US-PGPUB	2003/11/24 13:50

L6 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 6
TI Signal transduction by the c-Jun N-terminal kinase
AB A review with 77 refs. The c-Jun N-terminal kinase (JNK) group of mitogen-activated protein kinases (MAP kinases) is activated by exposure of cells to environmental stress and by the treatment of cells with cytokines. The mechanism of activation of JNK is mediated by dual phosphorylation within kinase subdomain VIII on the motif **Thr-Pro-Tyr**. This phosphorylation is mediated by the MAP kinase kinases MKK4 and MKK7. These MAP kinase kinases serve as signaling mols. that integrate a wide array of stimuli into the activation of the JNK signaling pathway. Studies of the physiol. function of JNK have been facilitated by the mol. genetic anal. of JNK signaling in Drosophila and by the creation of mice with targeted disruption of components of the JNK pathway. These studies demonstrate that the JNK pathway regulates AP-1 (activator protein-1) transcriptional activity in vivo and indicate that JNK is required for embryonic morphogenesis, the regulation of cellular proliferation and apoptosis, and the response of cells to immunol. stimuli.
SO Biochemical Society Symposia (1999), 64(Cellular Responses to Stress), 1-12
CODEN: BSSYAT; ISSN: 0067-8694
AU Davis, Roger J.

FILE 'CAPLUS, MEDLINE, BIOSIS, CA, SCISEARCH, EMBASE' ENTERED AT 13:53:06 ON 24 NOV 2003

L1	8692	S COMPETIT? (S) IMMUNOASSAY
L2	19	S THR-X-TYR
L3	0	S L1 AND L2
L4	4	DUPLICATE REM L2 (15 DUPLICATES REMOVED)
L5	67	S THR-PRO-TYR
L6	16	DUPLICATE REM L5 (51 DUPLICATES REMOVED)
L7	0	S L6 AND IMMUNOASSAY
L8	0	S JNK SAME ANTIBOD?
L9	1336	S JNK? (S) ANTIBOD?
L10	4538	S MKK?
L11	101	S L9 AND L10
L12	34	DUPLICATE REM L11 (67 DUPLICATES REMOVED)
L13	277292	S IMMUNOASSAY
L14	2	S L12 AND L13

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     FILE 'CAPLUS, MEDLINE, BIOSIS, CA, SCISEARCH, EMBASE' ENTERED AT 13:53:06
     ON 24 NOV 2003
L1          8692 S COMPETIT? (S) IMMUNOASSAY
L2            19 S THR-X-TYR
L3             0 S L1 AND L2
L4             4 DUPLICATE REM L2 (15 DUPLICATES REMOVED)
L5            67 S THR-PRO-TYR
L6            16 DUPLICATE REM L5 (51 DUPLICATES REMOVED)
L7             0 S L6 AND IMMUNOASSAY
L8             0 S JNK SAME ANTIBOD?
L9          1336 S JNK? (S) ANTIBOD?
L10         4538 S MKK?
L11          101 S L9 AND L10
L12           34 DUPLICATE REM L11 (67 DUPLICATES REMOVED)
L13       277292 S IMMUNOASSAY
L14            2 S L12 AND L13

     FILE 'STNGUIDE' ENTERED AT 14:04:41 ON 24 NOV 2003
L15            0 S THREONINE OR SERINE

     FILE 'CAPLUS, MEDLINE, BIOSIS, CA, SCISEARCH, EMBASE' ENTERED AT 14:06:59
     ON 24 NOV 2003
L16       466604 S THREONINE OR SERINE
L17        21548 S L16 (W) KINASE
L18           17 S L17 (W) SUBSTRATE
L19           11 DUPLICATE REM L18 (6 DUPLICATES REMOVED)
L20         2505 S L17 AND SUBSTRATE
L21      2873095 S ANTIBOD?
L22          382 S L20 AND L21
L23           13 S L22 AND COMPETIT?
L24            4 DUPLICATE REM L23 (9 DUPLICATES REMOVED)
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L3 ANSWER 10 OF 26 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 9

TI Immunoassay to determine Cyclin dependent kinase 5 activity by detection of disabled 1 protein phosphorylation

AB The present invention is based on the discovery that the Disabled 1 protein (Dab1) is a substrate for Cyclin dependent kinase 5 (Cdk5) activity, and is selectively phosphorylated by Cdk5. An assay to det. Cdk5 activity by detection of Dab1 phosphorylation on serine amino acids that are selectively phosphorylated by Cdk5 is provided. The invention further provides an antibody and screening kit to det. Cdk5 activity, a method for detecting a neurol. disorder by detg. Cdk5 activity, a method of screening for compds. that increase or decrease Cdk5 activity and a method for treating a neurol. disorder with such a compd.

SO U.S. Pat. Appl. Publ., 14 pp.
CODEN: USXXCO

IN Curran, Thomas; Keshvara, Lakhu

US 2002172990
WO 2003070879

DATE NO GOOD

L24 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3

TI Development and Validation of a **Competitive** AKT Serine/ **Threonine Kinase** Fluorescence Polarization Assay Using a Product-Specific Anti-phospho-serine **Antibody**

AB A **competitive** fluorescence polarization (FP) assay has been developed for the serine/**threonine kinase**, AKT. The FP assay has been formatted in a 384-well microtiter plate and automated using a pipetting workstation with performance suitable for high-throughput screening. The assay design utilizes a fluorescent phosphorylated peptide complexed to a product-specific anti-phospho-serine **antibody**. When unlabeled **substrate** is phosphorylated, by the kinase, the product competes with the fluorescent phosphorylated peptide for the **antibody**. The fluorescent phosphorylated peptide is then released from the **antibody** into soln. resulting in a loss in polarization signal. Seven fluorescent phosphorylated peptides and 19 **antibodies** were evaluated for this assay. RARTSpSFAEPGK-Fl peptide and anti-phospho-GSK-3.alpha. Ser21 **antibody** gave the best affinity and change in polarization signal. The apparent kinetic consts. were calcd. for the FP assay and were consistent with reported values. The FP assay was validated with known inhibitors and the results compared to a radioactive Flashplate transfer assay, utilizing [33P]ATP and a biotinylated **substrate**, also developed in our lab. The IC50 values generated were comparable between the two methods suggesting the **competitive** FP assay and Flashplate assay have similar sensitivities and abilities to identify inhibitors during screening. (c) 2001 Academic Press.

SO Analytical Biochemistry (2001), 299(1), 45-53
CODEN: ANBCA2; ISSN: 0003-2697

AU Turek, Tammy C.; Small, Eliza C.; Bryant, Robert W.; Hill, W. Adam G.

L3 ANSWER 22 OF 26 MEDLINE on STN

TI Immunoassay protocol for quantitation of protein kinase activities.

AB Quantitation of at least two orders of magnitude of kinase enzyme concentration is achieved with detection of less than 0.1 U/well of src kinase activity (Fig. 3). A comparison between a sequential protocol, in which biotinylated peptide substance is captured prior to incubation with the **kinase** enzyme, and a simultaneous protocol, in which peptide capture and the **kinase** reaction proceed concurrently, demonstrates that the simpler simultaneous protocol provides similar detection sensitivity. these have also been demonstrated with 0.1 microM peptide substrate in a protein **kinase** A assay.5 Quantitation of protein **kinase** activity with chemiluminescent detection has been demonstrated with several different protein kinases, including both tyrosine and **serine**/threonine kinases.5 An **immunoassay** format provides high sensitivity and can be performed under conditions that most closely mimic physiological substrate and ATP concentrations with chemiluminescent detection. This assay format is also automated easily for use in high-throughput screening.

SO METHODS IN ENZYMOLOGY, (2000) 305 410-6.
Journal code: 0212271. ISSN: 0076-6879.

AU Mosier J; Olesen C E; Voyta J C; Bronstein I

near
QP601.L72

L3 ANSWER 1 OF 26 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1

TI Identifying modulators for serine/threonine kinases using phospho-specific antibodies and kinase proteins fused with substrate proteins

AB A method is provided for identifying a modulator for serine/threonine kinase employing expression of fusion proteins of a kinase substrate, such as p53, and the serine/threonine kinase. The fusion protein between a substrate protein and a serine/threonine kinase is expressed is a cell, the cell incubated with a candidate modulator, and the level of phosphorylation of the substrate detd. Phosphorylation may be detd. in may ways, including Western blotting and ELISA using phospho-specific antibodies. Protein p53 is chosen as substrate within the fusion protein construct based on the fact that (1) p53 can be phosphorylated by multiple kinases such as Chk1/2, protein kinase A, and JNK kinase; (2) within p53 there are several phosphorylation sites; and (3) antibodies are com. available which recognize a particular phosphorylation site within p53. Preferred kinases can be derived from p38, JNK3, SGK, PLK1, YAK3, MAPKAPK2, MYT1, CDK5, ROCK1/2, and Chk1.

SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2

IN Suda, Mikiya; Shibahara, Megumi

WO 2003087394

L4 ANSWER 22 OF 23 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 20

TI A serine/threonine protein kinase that phosphorylates the N-terminal activation domain of the c-jun protein

AB An isolated 46 kDa (by reducing SDS-PAGE) protein (JNK) with a serine/threonine kinase activity that phosphorylates the c-Jun N-terminal activation domain and methods of detecting the protein are described. CDNAs encoding the protein are also described. JNK phosphorylates c-Jun N-terminal activation domain which affects gene expression from AP-1 sites. Proteins binding c-jun were identified by affinity chromatog. against immobilized c-jun and a c-jun kinase activity was detected and characterized. The binding of the kinase to c-jun was strong with most of the complex stable to NaCl 2M. The roles of the protein in c-jun activation, its role in the interaction of c-jun and c-Ha-ras proteins and in T-cell activation are studied.

SO PCT Int. Appl., 142 pp.
CODEN: PIXXD2

IN Karin, Michael; Davis, Roger; Hibi, Masahiko; Lin, Anning; Derijard, Benoit

WO 9503323

US 5534426

US 6014745

L3 ANSWER 2 OF 26 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2

TI Antibody specific to **serine**/threonine protein **kinase** for **immunoassay**

AB Provided are antibodies for detecting genomic system mediated by serine threonine protein kinase signalling pathway. The antibodies are prepd. in non-human warm-blooded animals such as rabbits using peptide of the protein kinase VIB domain conjugated with carrier protein (e.g. keyhole limpet hemocyanin) as immunogen. The antibodies are useful for immunodetection of Ca+2/calmodulin-dependent protein kinase CaMKII and CaMKIV, protein kinase A, protein kinase C, MAP kinase, MEK kinase, etc.

SO Jpn. Kokai Tokkyo Koho, 7 pp.
CODEN: JKXXAF

IN Shigesato, Yasushi; Itaru, Yoshiro; Yumoto, Noboru; Ishida, Atsuhiko; Kinoshita, Satoshi; Kameshita, Isamu

serine/threonine kinase

ANSWER 24 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Development and validation of a competitive AKT serine/threonine kinase fluorescence polarization **assay** using a product-specific anti-phospho-serine antibody.

AB A competitive fluorescence polarization (FP) **assay** has been developed for the serine/threonine kinase, AKT. The FP **assay** has been formatted in a 384-well microtiter plate and automated using a pipeting workstation with performance suitable for high-throughput screening. The **assay** design utilizes a fluorescent phosphorylated peptide complexed to a product-specific anti-phospho-serine **antibody**. When unlabeled substrate is phosphorylated, by the kinase, the product competes with the fluorescent phosphorylated peptide for the **antibody**. The fluorescent phosphorylated peptide is then released from the **antibody** into solution resulting in a loss in polarization signal. Seven fluorescent phosphorylated peptides and 19 **antibodies** were evaluated for this **assay**. RARTSpSFA-EPGK-F1 peptide and anti-phospho-GSK-3.alpha. Ser21 **antibody** gave the best affinity and change in polarization signal. The apparent kinetic constants were calculated for the FP **assay** and were consistent with reported values. The FP **assay** was validated with **known** inhibitors and the results compared to a radioactive Flashplate transfer **assay**, utilizing [(33)P]ATP and a biotinylated substrate, also developed in our laboratory. The IC(50) values generated were comparable between the two methods suggesting the competitive FP **assay** and Flashplate **assay** have similar sensitivities and abilities to identify inhibitors during screening. .COPYRGT. 2001 Elsevier Science.

SO Analytical Biochemistry, (1 Dec 2001) 299/1 (45-53).
Refs: 29
ISSN: 0003-2697 CODEN: ANBCA2

AU Turek T.C.; Small E.C.; Bryant R.W.; Hill W.A.G.